we present data defining the developmental and molecular consequences that result from epiblast specific disruption of YY1 function *in vivo*.

doi:10.1016/j.ydbio.2011.05.397

Program/Abstract #436
Cloning and functional study of Nanog in zebrafish
Jing Tian, Serene C. Chng, JunXian Ong, Bruno Reversade
*Institute of Medical Biology, A*star, Singapore, Singapore*

Nanog encodes for a homeobox-containing transcription factor critical for in vitro self-renewal of undifferentiated embryonic stem cells. Together with Oct4, Nanog assembles, in vivo, a transcriptional circuitry that ensures pre-implantation development in mammals. Here we report the cloning of the homolog of the mammalian nanog gene in Danio rerio and study its expression and function during fish early embryogenesis. Nanog mRNA was found to be maternal supplied in oocytes and ubiquitously expressed thereafter until gastrulation ends. In MZ spg embryos, which lack maternal and zygotic Oct4 activity, nanog expression was unaltered. Functional studies using MO-based knockdown and ZFN-mediated knockout suggest that nanog regulates blastomere division and germ layer patterning in zebrafish embryos.

doi:10.1016/j.ydbio.2011.05.398

Program/Abstract #437
Myogenin is expressed during primary myogenesis in *Xenopus*
Christina D. Young[a], Susan C. Howard[a], Paul R. Mueller[b]
[a]*University of Texas at San Antonio, San Antonio, TX, USA*
[b]*University of Texas At San Antonio Department of Biology, San Antonio, TX, USA*

Muscle development is dependent on four "master regulators" (MyoD, Myf5, MRF4, and myogenin) that are collectively known as the Muscle Regulatory Factors (MRFs). The MRFs are all members of a related family of basic helix-loop-helix (bHLH) transcription factors that promote myogenesis. In vitro, any one of these factors can convert a non-muscle cell into the muscle lineage. In vivo, the role of the MRFs in myogenesis is more complex. This complexity arises from the spatially and temporally restricted patterns of MRF expression, and the partial redundancy of MRF function. In mouse models, only myogenin is absolutely required, as the loss of any other MRF can be compensated by one or more of the other MRFs. Like other vertebrates, *Xenopus* expresses all four MRFs. However, while MyoD, Myf5, and MRF4 are expressed during primary myogenesis in the early *Xenopus* embryo, it has been reported that myogenin is not expressed until secondary myogenesis when the tadpole undergoes metamorphosis to become an adult frog. This situation leaves open to speculation how myogenesis takes place in the early *Xenopus* embryo and tadpole in the absence of myogenin. We have re-examined this problem and find that myogenin is in fact expressed in the early *Xenopus* embryo when primary myogenesis is taking place. Using qPCR we find that myogenin transcripts are detectable from mid-neurulation stages onward. In situ hybridization shows that this expression is in the pre-somitic mesoderm of the early embryo and the somites and migrating muscle precursors of the tadpole. This result raises the possibility that, as in other vertebrate embryos, *Xenopus* tadpoles also require myogenin for myogenesis.

doi:10.1016/j.ydbio.2011.05.399

Program/Abstract #438
Loss of a CITED-family transcription coactivator results in muscular atrophy and impaired motility
Gnanapackiam Sheela Devakanmalai[a], Ertugrul M. Ozbudak[b]
[a]*Albert Einstein College of Medicine Genetics Department, Bronx, NY, USA*
[b]*Albert Einstein College of Medicine Genetics, Bronx, NY, USA*

In vertebrates, muscle development occurs through the sequential segmentation of mesoderm tissue into repetitive structures called somites. Recent spatiotemporal microarray studies have identified novel, uncharacterized transcription factors which might be involved in muscle differentiation and/or fiber-type specification. Cited3 is one such novel gene encoding a transcriptional cofactor. Cited3 is expressed in the oxidative fiber precursors (slow muscle), brain, neural crest cells, branchial arches and hatching gland of zebrafish embryos and we identified that its expression is regulated by Hedgehog signaling. In this study, Cited3 expression was knocked down in zebrafish embryos by injecting morpholino oligonucleotides that blocks the splicing of Cited3. Reduction in Cited3 expression resulted in morphological abnormalities such as hatching gland defects and tail curvature. Slow fiber-specific immunostaining shows a significant reduction in expression of slow myosin heavy chain, number and width of the fiber indicating impairment in slow fiber myogenesis in Cited3 morphants. We also found that reduction of Cited3 expression slows earlier undulating movements and later escape mechanism eventually leading to total immobility at 5 days-post-fertilization. Tunnel Assay indicates the occurrence significantly large number of apoptotic cells in the muscle of the morphants. Finally, overexpression of Cited3 mRNA could significantly rescue Cited3 morphants phenotype. In situ hybridization for various muscle-specific genes reveals the gene regulatory network that is controlled by Cited3. This is the first report that Cited3 functions in myofibrillogenesis of oxidative/slow-twitch muscle fibers and its absence can lead to muscle atrophy and immobility.

doi:10.1016/j.ydbio.2011.05.400

Program/Abstract #439
Foxa1 and Foxa2 in the intervertebral disk
Jennifer Maier, Yin Ting Lo, Brian Harfe
Gainesville, FL, USA

The intervertebral disk (IVD) is composed of an outer annulus fibrosus (AF), and an inner, gel-like nucleus pulposus (NP). The NP is derived from the notochord in mice. Chronic back pain as a result of disk degeneration is a common health condition for which there are limited treatment options. Current treatments involve surgery or painkillers but do not address the problem of disk degeneration and are not very effective. Little is known about the mechanisms of IVD development and degeneration; this information could lead to improved treatments. To study disk development, we are looking at the forkhead box (Fox) genes, which are expressed in the notochord. They are also expressed in many other tissues and are vital to development and post natal life. Foxa1 and Foxa2 genes have been well-studied in the endoderm, but not in the notochord. Foxa2 null mice die in utero lacking a notochord. Cre alleles have been used to ablate Foxa2 in the endoderm. These conditional alleles have also been used with a Foxa1 null allele to make double knockouts. We used these alleles with an inducible ShhERT2cre line to remove Foxa2 in tissues where Sonic hedgehog is expressed in E7.5 mouse embryos. Fate-mapping with the Rosa26 reporter allele was done. Mice null for Foxa1 and lacking Foxa2 in Shh-expressing cells appear to have a severely deformed NP and a shortened tail. Fate-mapping in these mice suggests defects in the migration of notochord cells to the NP.

There also appears to be massive cell death in the somites and notochord of double mutants. We have also examined gene expression using RNA in situ hybridization to look at the effect of the deletion of Foxa1 and Foxa2 on other genes required for notochord formation and NP development. Study of the role of Foxa family action in IVD development may provide insight into new treatments for disk degeneration.

doi:10.1016/j.ydbio.2011.05.401

Program/Abstract #440
Differential requirement of ZIC3 function in cardiac development and X-linked heterotaxy
Zhengxin Jiang[a], Lirong Zhu[b], Lingyun Hu[b], Robia Pautler[b], Monica Justice[b], John Belmont[b]
[a]*Baylor College of Medicine Dept of Molecular & Human Genetics, Houston, TX, USA*
[b]*Baylor College of Medicine, Houston, TX, USA*

Heterotaxy, contributing to ~5% of congenial heart defects (CHD), arises from abnormal left-right patterning. Mutations of ZIC3 gene (Zinc finger protein of cerebellum 3) are associated with human X-linked heterotaxy. A mouse model with targeted disruption of Zic3 exhibited ~75% early lethality, and recapitulated the phenotype seen in human patients. However, it is not known whether ZIC3 is required in a single developmental field or whether it has pleiotropic roles in multiple developmental processes, and the detailed mechanism remains elusive. To address these questions, we generated a conditional allele of the Zic3 gene by flanking its 1st exon with loxP sites. Sox2-cre, Wnt1-cre and T-cre lines were used to delete Zic3 in epiblast, neural crest and mesoderm, respectively. Deletion of Zic3 in epiblast and mesoderm, but not in neural crest, led to ~50% early lethality. Examination of epiblast conditional embryos by microscopy revealed multiple CNS and neural tube defects similar to the null embryos. But these defects were not found in mesoderm or neural crest conditional embryos, suggesting that Zic3's function in CNS development likely remains intact in these mutants. MRI scanning of Zic3 epiblast and mesoderm conditional embryos also uncovered multiple heterotaxy related visceral abnormalities. Gene expression analysis by microarray in the hearts of embryos at 15.5 dpc revealed a similar expression pattern between Zic3 epiblast conditional and null males, which was significantly different from control males. Perturbed expression of several cardiac genes and direct targets of Zic3 suggested that Notch, BMP and TGF-β signaling might be affected, and requires further investigation.

doi:10.1016/j.ydbio.2011.05.402

Program/Abstract #441
Hox genes control the axis elongation process in chicken embryo
Nicolas Denans[a], Tadahiro Iimura[b], Olivier Pourquie[c]
[a]*IGBMC Olivier Pouquie Lab, Illkirch, France*
[b]*Tokyo Medical and Dental University International Research Center for Molecular Science in Tooth and Bone Diseases Department of Molecular Pathology, Tokyo, Japan*
[c]*IGBMC Inserm U964, CNRS (UMR 7104), Université de Strasbourg, Illkirch, France*

The vertebae's precursors, the somites, are formed periodically by the segmentation of the presomitic mesoderm (PSM) which forms by progressive cell deposition from a posterior growth zone. The number of somites is precisely defined for any given species but varies widely from one species to another. In order to maintain a precise number of somites, the body axis elongation has to be tightly controlled. Indeed, using time-lapse imaging of developing chicken embryos we observed that elongation process slows down few hours before the termination of the axis. We previously showed that a gradient of random cell motility within the PSM is implicated in axis elongation (Benazeraf et al., 2010). However the precise control of how the elongation will slow down to define the axis length remains unknown. To address this issue, we used the electroporation technique coupled to time-lapse imaging of developing chicken embryos. Using these techniques we show that cell motility in the PSM decreases progressively at the end of axis elongation. Nevertheless this decrease in cell motility is not sufficient to explain the slowing down of axis elongation. We previously showed that Hox genes are expressed in a collinear fashion in the PSM precursors and control the timing of ingression of the PSM precursors (Iimura and Pourquie 2006). Overexpression of different Hox genes alters body axis elongation. This effect takes place in part by controlling cell motility in the posterior PSM but mainly by regulating the flux of cells ingressing in the PSM. Altogether we propose a new mechanism explaining how the collinear expression of the Hox genes regulates the length of the body axis.

doi:10.1016/j.ydbio.2011.05.403

Program/Abstract #442
Role of 5′HOXD genes in the endochondral ossification
Carmen González-Martín, Carlos Garrido-Allepuz, Marian Ros
CSIC, Santander, Spain

Mutations with gain and loss-of-function of Hoxd genes present notably osteogenic defects indicating the involvement of these genes in the endochondral ossification. To clarify the role of Hoxd genes in endochondral ossification, we have analyzed the osteochondrogenic program in the autopod of mice lacking Hoxd11 to 13 (HoxdDel11–13/Del11–13), the animal model for the human synpolydactyly. This mutant is characterized by short and sometimes biphalangeal digits and by an extremely ossification delay. The maximum phenotypic defect occurs in the metacarpals/metatarsals that at birth lack the primary ossification center and collar bone. Ossification center the phalanges is partially abnormal and ventrally biased. During embryonic development Ihh and Runx2 expression is undetectable in the chondrocytes and perichondrium respectively, reflecting the abnormal organization and differentiation of the bone anlagen. The similarity of the phenotype with that of Ihh mutants prompted us to perform the compound Gli3; HoxdDel(11–13) mutant. Interestingly, removal of Gli3 from the HoxdDel(11–13) background rescued ossification in the hindlimb (metatarsals) but only partially in the forelimb (metacarpals). Our results support the involvement of Hoxd11–13 in the formation of the perichondrium and in the regulation of Ihh expression. Supported by grant BFU2008-00397 from the Spanish Ministry of Science and Innovation.

doi:10.1016/j.ydbio.2011.05.404

Program/Abstract #443
HMGB factors are required for posterior digit development through integrating Shh, Wnt and BMP signaling pathways in the forelimb
Junji Itou[a], Noboru Taniguchi[b], Isao Oishi[c], Hiroko Kawakami[a], Martin Lotz[b], Yasuhiko Kawakami[d]
[a]*Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN, USA*